3. Physiological and therapeutic roles of taurine in the heart

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1.1. Introduction

Taurine is the most abundant free amino acid in the heart. However, unlike the classical β-amino acids, taurine is a β-amino acid with the acid moiety being a sulfonic acid group rather than a carboxyl group; the lack of a carboxyl group excludes the involvement of taurine in peptide bond formation. Nonetheless, there is abundant evidence that taurine is an essential nutrient in certain species [1, 2] and semi-essential in other species [3]. Because of its abundance in the heart, there has been renewed interest in its role in contractile function. The initial evidence that taurine is essential for normal cardiovascular function was uncovered using nutritional and taurine transport inhibitor models of taurine depletion. While the nutritional deficiency studies have established a link between taurine depletion and the development of a cardiomyopathy, the nutritional model is difficult to reproduce. By comparison, the taurine transport inhibitor model is easy to reproduce but the interpretation of the results is problematic because of the side effects of the transport inhibitors. Because the heart depends upon an extracardiac source of taurine to maintain its large

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intracellular stores, the recent development of two readily reproducible taurine transporter knockout models dramatically improves the chances of definitively establishing the key physiological functions of taurine in the heart.

In addition to the physiological actions of taurine, the β-amino acid exhibits a number of important pharmacological actions. These effects are observed when the heart is exposed to high extracellular concentrations of taurine. These effects have generated considerable interest in the field because chronic taurine administration is largely associated with cytoprotection and modulation of important cellular events, such as contractile function.

The present review discusses several putative physiological actions of taurine, including maintenance of contractile function, antioxidant activity, membrane stabilization, osmoregulation and regulation of protein phosphorylation. Also to be discussed in the review, are the therapeutic implications of taurine’s actions relative to several cardiovascular diseases. To date, the only accepted therapeutic use of taurine is in the treatment of heart failure. The present review discusses other cardiovascular conditions that might benefit from taurine therapy.

1.2. Physiological functions of taurine

1.2.1. Role of taurine in maintenance of normal contractile function of heart

The maintenance of high myocardial taurine levels is required for normal contractile function. Several lines of evidence lead to the conclusion that severe reductions in cellular taurine content underlie the development of a dilated cardiomyopathy [4-10]. First, the taurine deficient cardiomyopathy in cats is nutritionally based. Pion et al. [4] found that the taurine deficient cardiomyopathy only develops in cats fed commercial diets that induce low plasma taurine levels or maintained for years on a purified diet containing reduced concentrations of taurine. Second, the taurine deficient cardiomyopathy is reversible, as evidenced by the recovery of left ventricular function in taurine deficient cats fed a diet rich in taurine [4, 11]. Furthermore, the incidence of feline dilated cardiomyopathy has dramatically declined since the supplementation of commercial cat food with taurine [11, 12]. Third, rodents, which normally synthesize enough taurine in the liver to maintain high myocardial taurine levels, develop both a cardiomyopathy and myopathy when the taurine transporter is genetically abolished [10].

The taurine deficient cardiomyopathy is characterized by both systolic and diastolic dysfunction. A study of 37 prospective cats suffering from a dilated cardiomyopathy revealed defects in left ventricular end-diastolic dimension and
left ventricular end-systolic internal dimension [12]. In a subsequent study, Novotny et al. [7-9] found that cats fed purified diet containing inadequate taurine levels (0.1%) also developed a cardiomyopathy characterized by systolic dysfunction (left ventricular end-systolic diameter, fractional shortening, +dP/dt) and diastolic dysfunction (left ventricular end-diastolic diameter, left ventricular chamber compliance, -dP/dt) although the systolic defects were the more prominent. Some of the cats in both studies exhibited systolic dysfunction coupled with increased left ventricular chamber size, conditions consistent with the development of a dilated cardiomyopathy.

Taurine deficient cardiomyopathies are not unique to cats; they also develop in nutritionally compromised dogs and fox [5, 6] and in mice lacking exons 2-4 of the taurine transporter [10]. However, mice lacking only exon 2 of the transporter do not develop a cardiomyopathy despite the presence of a severe myopathy [13]. The basis for the difference in severity between the two taurine transporter knockout models is unclear. It has been suggested that variables, such as age and diet, could contribute to the susceptibility of the mutated myocardium to taurine depletion. Thus, the development of the taurine deficient cardiomyopathy is in all likelihood complex and unlikely to be attributed solely to taurine deficiency.

1.2.2. Ion transport and development of taurine deficient cardiomyopathy

Figure 1 illustrates the events involved in excitation-contraction coupling of the heart. When the heart is stimulated, the cardiomyocyte depolarizes allowing calcium to enter the cell. The rise in [Ca\(^{2+}\)]\(_i\) triggers the release of calcium from intracellular sarcoplasmic reticular (SR) calcium stores, an event referred to as Ca\(^{2+}\)-induced Ca\(^{2+}\) release. When the [Ca\(^{2+}\)]\(_i\) exceeds the K\(_d\) for the troponin calcium binding sites, the muscle proteins undergo conformational changes in which the myosin Ca\(^{2+}\) ATPase is activated and the actin-myosin bridges form. Relaxation occurs when the SR Ca\(^{2+}\) ATPase pumps sufficient calcium into the SR vesicles to allow the dissociation of calcium from the troponin binding sites. Additional calcium leaves the cell via the Na\(^+\)/Ca\(^{2+}\) exchanger.

A major cause of contractile dysfunction in the failing heart is impaired delivery of calcium to and removal of calcium from the muscle proteins, a process referred to as excitation-contraction coupling [14]. One of the key calcium transporters involved in excitation-contraction coupling is the SR Ca\(^{2+}\) ATPase, which catalyzes a series of enzyme reactions ultimately leading to the uptake of calcium by the sarcoplasmic reticulum (Figure 1). Elevations in SR Ca\(^{2+}\) ATPase activity enhance myocardial relaxation through reductions
Figure 1. Excitation-contraction coupling of the heart. Upon stimulation of the cardiomyocyte, the membrane depolarizes and calcium enters the cell via the L-type Ca\(^{2+}\) channel. The incoming Ca\(^{2+}\) triggers the release of Ca\(^{2+}\) from the sarcoplasmic reticulum, a process referred to as Ca\(^{2+}\)-induced Ca\(^{2+}\) release. As \([\text{Ca}^{2+}]_i\) rises, sufficient Ca\(^{2+}\) becomes available to the troponin Ca\(^{2+}\) binding sites, an event that initiates contraction. Relaxation occurs when Ca\(^{2+}\) is pumped back into the sarcoplasmic reticulum by the SR Ca\(^{2+}\) ATPase. Some Ca\(^{2+}\) also leaves the cell via the Na+/Ca\(^{2+}\) exchanger. RYR – ryanodine receptor.

in \([\text{Ca}^{2+}]_i\) during diastole leading to the release of calcium from troponin binding sites. Taurine affects SR Ca\(^{2+}\) ATPase activity but only through indirect mechanisms. When enriched SR preparations are exposed acutely to medium containing taurine there is no change in SR Ca\(^{2+}\) uptake [15-17]. However, drug-induced taurine depletion of the heart reduces SR Ca\(^{2+}\) pump activity [17]. Recently, Schaffer et al. [18] showed that the phosphorylation state of the SR phosphoprotein, phospholamban, is reduced in hearts of the taurine transporter knockout mouse. Because hearts overexpressing phospholamban or containing dephosphorylated phospholamban exhibit impaired SR Ca\(^{2+}\) uptake and myocardial relaxation [19], the prolongation of both the calcium transient and the contraction cycle in drug-induced taurine deficient hearts could be caused at least in part by the dephosphorylation of phospholamban [20].
The major determinant of $[\text{Ca}^{2+}]_i$ rise during systole is $\text{Ca}^{2+}$-induced $\text{Ca}^{2+}$ release. According to Punna et al. [21] the release of $^{45}\text{Ca}^{2+}$ from $^{45}\text{Ca}^{2+}$-loaded junctional sarcoplasmic reticular vesicles by medium containing calcium is promoted by 30 mM and 50 mM taurine. A related phenomenon, caffeine-induced, $\text{Ca}^{2+}$ release is also regulated by taurine [22]. Skinned fibers (fibers treated with detergent to permeabilize the cell membrane) placed in medium lacking taurine release moderate amounts of $\text{Ca}^{2+}$ from the sarcoplasmic reticulum in response to caffeine. However, addition of 30 mM taurine to the medium increases caffeine-induced $\text{Ca}^{2+}$ release when the sarcoplasmic reticulum is preloaded with low amounts of calcium, but when the calcium load of the sarcoplasmic reticulum is high caffeine-induced $\text{Ca}^{2+}$ release decreases. Because $\text{Ca}^{2+}$-induced $\text{Ca}^{2+}$ release can occur spontaneously in skinned fiber preparations loaded with large amounts of calcium the significance of the taurine-mediated decrease in caffeine-induced $\text{Ca}^{2+}$ release in these preparations is unclear. Interpretation of the skinned fiber data is further complicated by the observation that not only does taurine promote $\text{Ca}^{2+}$ induced $\text{Ca}^{2+}$ release from the sarcoplasmic reticulum but L-type $\text{Ca}^{2+}$ channel flux also affects $\text{Ca}^{2+}$ induced $\text{Ca}^{2+}$ release in the intact cell [23]. Because multiple factors affect $\text{Ca}^{2+}$ induced $\text{Ca}^{2+}$ release in the intact cell, it is not surprising that the effect of taurine on $\text{Ca}^{2+}$-induced $\text{Ca}^{2+}$ release in intact cardiomyocytes still remains unclear. Eley et al. [24] concluded that only a fraction of the contractile defect of the drug-induced taurine depleted heart can be attributed to impaired SR $\text{Ca}^{2+}$ handling. However, the model of taurine deficiency used in that study depended upon the taurine transport inhibitor, guanidinoethane sulfonate, to reduce taurine levels. Because guanidinoethane sulfonate only decreases taurine levels by ~50-70% (in contrast to > 99.9% in the taurine transporter knockout mouse) and exhibits serious side effects, such as depression of creatine phosphate content [25], the interpretation of the study is problematic. Moreover, an earlier study by the same group [26], reported a significant reduction in the fraction of calcium that recirculates through the sarcoplasmic reticulum of the guanidinoethane sulfonate-induced taurine depleted heart. Thus, the taurine transporter knockout model offers the best opportunity for clarifying the effects of taurine on SR $\text{Ca}^{2+}$ release.

Another potential site of taurine action is the $\text{Na}^+$/Ca$^{2+}$ exchanger, whose direction of flux depends on the $[\text{Ca}^{2+}]_i/[\text{Ca}^{2+}]_o$ and $[\text{Na}^+]/[\text{Na}^+]_o$ ratios. However, the effects of taurine on $\text{Na}^+$/Ca$^{2+}$ exchange activity remain an area of active debate. While Katsube and Sperelakis [27] found no effect of acute taurine exposure on the activity of the $\text{Na}^+$/Ca$^{2+}$ exchanger, chronic taurine depletion leads to a decrease in the activity of the exchanger [17].
mechanism underlying the taurine-linked depression of Na\(^+\)/Ca\(^{2+}\) exchanger activity has not been definitively established. Nonetheless, Hamaguchi et al. [28] found that taurine modulates the phosphatidylcholine and phosphatidylethanolamine content of the membrane, which in turn affects the activity of the Na\(^+\)/Ca\(^{2+}\) exchanger. Studies utilizing the intact heart have also failed to clarify the role of the Na\(^+\)/Ca\(^{2+}\) exchanger in the development of contractile defects of the taurine deleted heart. While Lake [26] raised the possibility that reductions in SR Ca\(^{2+}\) pump activity of the taurine deficient heart might redirect more calcium extrusion to the Na\(^+\)/Ca\(^{2+}\) exchanger, Eley et al. [24] relied on force-interval relationships to conclude that efflux of calcium from the cell is not appreciably altered by taurine depletion. Because changes in Na\(^+\)/Ca\(^{2+}\) exchanger activity can lead to alterations in the size of the myocardial Ca\(^{2+}\) pool and in contractile function, further studies utilizing the taurine transporter knockout heart to evaluate taurine-mediated regulation of the Na\(^+\)/Ca\(^{2+}\) exchanger are warranted.

In addition to the modulation of calcium movement, taurine may also affect the movement of potassium within the cardiomyocyte. Sawamura et al. [29] reported that pharmacological concentrations of extracellular taurine prolong the action potential of hearts exposed to [Ca\(^{2+}\)]\(_o\) of 0.8 mM but shorten the action potential at [Ca\(^{2+}\)]\(_o\) of 3.6 mM, effects they suggested might involve changes in potassium transport. In support of this idea, Satoh [30] found that pharmacological levels of taurine inhibit the delayed rectifier K\(^+\) current when the [Ca\(^{2+}\)]\(_i\) is fixed at 1.0 \(\mu\)M. Interestingly, taurine depletion is also associated with prolongation of the action potential and of the QT interval, an effect Lake [26] attributed in part to decreases in the delayed rectifier K\(^+\) current. However, Schaffer et al. [20] showed that drug-induced taurine depletion also prolongs the contraction cycle of the heart. Since the calcium transient of the taurine depleted cardiomyocyte is also prolonged, changes in calcium movement could also contribute to the prolongation of the action potential.

The taurine transporter located on the cell membrane is a taurine-Na\(^+\) symporter. Because of the link between taurine content and [Na\(^+\)]\(_i\) established by the symporter reaction, elevations in [Na\(^+\)]\(_i\) to concentrations exceeding 20 mM provoke an efflux of taurine from the cell [31]. Conversely, an increase in the transmembrane gradient for taurine is associated with a decrease in [Na\(^+\)]\(_i\) [32]. Collectively, these findings support a role for the taurine transporter in the movements of taurine and Na\(^+\) into and out of the cardiomyocyte. Not only does the movement of these osmolytes affect osmotic pressure but they also alter [Ca\(^{2+}\)]\(_i\), factors which have a profound influence on hearts subjected to major pathological insults, such as ischemia or the development of heart failure (see discussion below).
1.2.3. Apoptosis and development of taurine deficient cardiomyopathy

A key process in the development of overt heart failure is a series of events resulting in changes in cardiomyocyte and ventricular size and shape, which collectively are referred to as ventricular remodeling. Multiple factors contribute to the development of ventricular remodeling, with one of the more important factors being apoptotic cell death. Because taurine is a major contributor to apoptosis it follows that taurine must also be a modulator of ventricular remodeling. Taurine regulates apoptosis at several steps in the death cascade. During the course of the apoptotic cascade, cells undergo an exaggerated regulatory volume decrease in which taurine is transported out of the cell. However, if the cell is preloaded with taurine prior to exposure to an apoptotic stimulus, the apoptotic cascade does not progress beyond the cell shrinkage step [33]. It has been reported that taurine can also interfere with the formation of the apoptosome [34]. However, the most widely discussed anti-apoptotic action of taurine focuses on the regulation of the mitochondrial permeability transition. In 1979, Haworth and Hunter [35] described a phenomenon, which they called “calcium-induced membrane transition in mitochondria.” This transition was characterized by matrix swelling and the release of substances from the mitochondria. Today, the membrane transition is recognized as a characteristic of the mitochondrial permeability transition, which in turn initiates apoptosis through the release of pro-apoptotic factors from the mitochondria (Figure 2). One of the key triggers of the mitochondrial permeability transition is calcium overload, whose effect is enhanced by oxidative stress [36]. Because taurine is a modulator of both mitochondrial oxidative stress and cellular calcium (see sections on ion transport and oxidative stress), it is not surprising that taurine regulates both the mitochondrial permeability transition and apoptosis.

1.2.4. Antioxidant activity of taurine

Arouma et al. [37] have clearly shown that taurine is incapable of directly scavenging the classical reactive oxygen species (ROS). Nonetheless, there is mounting evidence that taurine serves as an indirect antioxidant, although the mechanism underlying the antioxidant activity of taurine remains unclear. It has been proposed that the only ROS that is directly neutralized by taurine is HOCl, an oxidant generated by neutrophils [38]. The reaction between taurine and HOCl produces N-chlorotaurine, a product capable of killing cells although less effectively than HOCl. Thus,
Figure 2. Role of mitochondrial permeability transition in initiation of apoptosis.
Excessive levels of Ca2+ in combination with oxidative stress initiate formation of the mitochondrial permeability transition pore, a pathological channel that allows solute to leave the mitochondrial matrix and enter the cytosol. Pro-apoptotic factors, such as cytochrome c, enter the cytosol, when it associates with ATP and Apaf 1 to form an apoptosome. The apoptosome binds and activates caspase 9, which in turn activates caspase 3, an enzyme that cleaves important substrates within the cell, causing apoptotic cell death.

during inflammatory events, the neutralization of HOCl by taurine limits oxidative damage caused by neutrophils [38].

Recent evidence suggests that an improvement in mitochondrial status underlies the antioxidant activity of taurine. In 2002 Suzuki et al. [39] reported the existence of two novel taurine-containing uridines, 5-taurinomethyluridine and 5-taurinomethyl-2-thiouridine, in some of the mitochondrial tRNAs for leucine and lysine. In the case of tRNA\(^{\text{Leu(UUR)}}\) the modified uridine is situated
in the Wobble position of the tRNA [40]. When the taurine modification is absent from the Wobble position, the translation of the UUG codon, which binds to the tRNA\textsubscript{Leu(UUR)} anticodon, is reduced (Figure 3).

This overlying mechanism was the basis for suggesting that defects in the translation of the UUG codon mediate the decrease in the synthesis of mitochondria encoded proteins [39]. Because these mitochondrial proteins become incorporated into respiratory chain complexes, Schaffer \textit{et al.} [41] proposed that reductions in mitochondrial protein synthesis lead to a decrease in respiratory chain activity, reducing ATP synthesis and increasing superoxide generation. This occurs because electron transport is limited by reduced respiratory chain activity. Consequently, electrons are diverted from the electron transport chain to oxygen, forming in the process superoxide anion. According to this tRNA hypothesis, normal mitochondrial levels of taurine and 5-taurinomethyluridylidine are required to ensure proper respiratory chain activity. In turn, high rates of electron transport maximize coupling.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure3.png}
\caption{Localization of 5-taurinomethyluridylidine (\(\tau\text{m5U}\)) in the wobble position of tRNA\textsubscript{Leu}. (A) Structure of 5-taurinomethyluridylidine (\(\tau\text{m5U}\)). (B) Binding of tRNA\textsubscript{Leu} to mRNA. The anticodon (\(\tau\text{m5UAA}\)) for Leu on tRNA\textsubscript{Leu} binds to codons of UUG or UUA, with binding to UUG but not UUA adversely affected by the absence of the taurine conjugate.}
\end{figure}
between electron transport and ATP synthesis and minimize mitochondrial superoxide formation.

Recent work by Jong et al. [42] confirms that cellular taurine deficiency leads to a decrease in the levels of one of the mitochondrial encoded proteins, ND6, a protein whose mRNA contains extremely high UUG content. The reduction in ND6 levels could not be attributed to a decline in either mitochondrial taurine or 5-taurinomethyluridine content. One explanation for this apparent discrepancy relates to the $K_m$ of the mitochondrial taurine transporter. Because $K_m$ values of most membrane transporters are in the µM to low mM range, the two fold drop in cytosolic taurine content (i.e. from 20-30 mM to 10-15 mM) mediated by the taurine transport inhibitor, β-alanine, is unlikely to significantly affect the rate of taurine transport into the mitochondria. According to Jong et al. [42] a logical explanation for the finding is that β-alanine, a structural analogue of taurine, directly modifies uridine. The resulting β-alanine conjugated uridine interferes with the normal codon-anticodon interactions for leucine, thereby slowing the synthesis of ND6. Because complications can readily arise from the structural similarities of taurine and the taurine transport inhibitors, the real effects of taurine are masked in the drug-induced deficient cell. These complications are absent in the taurine transporter knockout mouse model, making it ideal for testing the tRNA hypothesis.

1.2.5. Membrane stabilizing activity of taurine

The concept that taurine stabilizes membranes is based on a study by Huxtable and Bressler [43], who found that exposure of isolated sarcoplasmic reticulum to taurine minimizes phospholipase C-mediated membrane damage. The mechanism underlying this phenomenon was never determined, however, the most likely mechanism involves a shielding of the phospholipids by taurine. Chovan et al. [44, 45] identified two types of taurine binding sites on the myocardial cell membrane, the high affinity sites representing the taurine transporter and the low affinity taurine binding sites implicated in the positive inotropic effect of taurine. Because the binding of taurine to the low affinity sites increases membrane calcium binding, Chovan et al. [44] proposed that taurine increases the amount of calcium available for contraction. In support of this notion it was found that interference with taurine binding simultaneously blocks calcium binding to the membrane and reduces contractile performance. Sebring and Huxtable [46] subsequently identified the low affinity binding sites as phospholipids, with phosphatidylcholine and phosphatidyl-ethanolamine exhibiting the highest capacity to bind taurine. Figure 4 shows the likely interaction of taurine with phosphatidyl-
Figure 4. Binding of taurine to phosphatidylethanolamine. Taurine forms an ionic interaction with phosphatidylethanolamine, with the sulfonic acid moiety and the amino group of taurine binding to the amino group and phosphate moiety of phosphatidylethanolamine, respectively.

Although the ionic interaction between taurine and the phospholipids alters some properties of the membrane, the most dramatic change in membrane function appears to involve taurine-mediated inhibition of phospholipid N-methyltransferase, an enzyme that catalyzes the conversion of phosphatidylethanolamine (PE) to phosphatidylcholine (PC) [28]. Because phosphatidylcholine contains a large head group (phosphocholine) while phosphatidylethanolamine contains a small head group (phosphoethanolamine), the two phospholipids assume very different structures in aqueous medium and within biological membranes. According to the fluid mosaic model of membrane structure, membranes are arranged as fluid
semipermeable bilayers. However, the structure of the membrane is not fixed, as it can undergo transient departures from the bilayer structure, a diversion that alters membrane functions such as transport, fusion and enzyme activity. Phospholipid N-methyltransferase, which alters the PC/PE ratio of the membrane, dramatically changes the frequency of phase transitions and induces localized environmental changes within the membrane. This occurs because PE facilitates the bilayer structure and preferentially localizes to the outer membrane leaflet while PC is preferentially localized to the inner leaflet of the membrane and transiently disrupts the bilayer structure [47].

1.2.6. Osmoregulatory activity of taurine in heart

Cardiomyocytes, like most cells, do not tolerate extreme alterations in cell size. Nonetheless, while most cells contain volume regulatory mechanisms to limit deviations in cell size, there is an ongoing debate whether mammalian cardiomyocytes possess effective volume regulatory mechanisms. Much of that debate focuses on the effects of a small membrane spanning protein known as phospholemman. As a member of the FXYD family of small membrane spanning proteins, phospholemman has attracted attention in the taurine field because it forms volume-sensitive channels that are fairly specific for anions and taurine [48]. Although a reduction in phospholemman expression decreases taurine efflux in astrocytes [49], cardiomyocytes of phospholemman knockout mice undergo osmotic swelling to the same degree as wild-type cells. Indeed, some investigators have failed to detect volume regulatory mechanisms in the mouse heart [50]. Nonetheless, various components of the regulatory volume decrease are present in the mammalian cardiomyocyte, including a rapid taurine efflux mechanism [51]. Moreover, the cardiomyocyte appears to retain certain components of the regulatory volume increase, such as hyperosmotic-mediated taurine uptake [52, 53]. These findings are supported by recent evidence that the promoter region of the taurine transporter contains a transcriptional tonicity-response element, which is involved in hyperosmotic stress-induced upregulation of the taurine transporter [54].

In contrast to mammalian cardiomyocytes, there is little doubt that chick and flounder cardiomyocytes undergo volume regulation [55, 56]. In fact, the identification of taurine as an osmoregulator originated with the work of Vislie and Fugelli [55], who found that flounder undergoing a transition from salt to fresh water experience declines in both plasma osmolality and myocardial taurine content. Although taurine transport was not directly examined in adapting flounder, isolated chick cardiomyocytes subjected to a hypoosmotic insult initially swell and then undergo a volume regulatory event that includes the extrusion of cations, anions and taurine from the cell [56].
Despite the debate centering on the significance of volume regulatory mechanisms in the mammalian heart, there is overwhelmingly support for the view that taurine transport is regulated by osmotic stress. The confusion surrounding these seemingly inconsistent findings has raised questions regarding the physiological relevance of taurine’s osmoregulatory activity in the mammalian heart. A key question to answer is whether the osmoregulatory activity of taurine in the mammalian heart serves some other important function besides regulation of cell size. One possible scenario is that the “osmoregulatory activity” of taurine actually contributes to cell survival. Pastukh et al. [57] have shown that mild hyperosmotic stress, including stress caused by either reductions in intracellular taurine content or elevations in extracellular taurine, activates survival pathways in the cardiomyocyte (Figure 5). This notion is consistent with recent report by Han and Chesney [58] showing that upregulation of the taurine transporter reduces the rate of renal apoptosis initiated by cisplatin.

![Figure 5. Activation of pro-survival pathways by osmotic preconditioning.](image)

**Figure 5. Activation of pro-survival pathways by osmotic preconditioning.** A bout of mild osmotic stress prior to ischemia or hypoxia activates the PI 3-kinase/Akt survival pathway. The active, phosphorylated form of Akt (p-Akt) inactivates caspase 9 and blocks the phosphorylation of glycogen synthase kinase-3β and BAD. It also stimulates the phosphorylation of eNOS and mdm2, the latter which blocks p53.

### 1.2.7. Modulation of protein phosphorylation by taurine

Taurine-mediated changes in osmotic stress and protein phosphorylation appear to be closely linked, in part because osmotic stress initiates protein
kinase-containing signaling pathways. One of the protein kinases linked to both osmotic stress and taurine-mediated osmoregulation is protein kinase C. Interestingly, only selective protein kinase C isoforms (PKCα, PKCε, and PKCζ) respond to hypoosmotic stress by undergoing intracellular translocation and activation [59, 60]. In the osmotic stress-mediated signaling pathway, these same PKC isoforms then stimulate NADPH oxidase, which is a major source of cytosolic superoxide. Cytosolic ROS in turn inhibit protein tyrosine phosphatase 1B, potentiating swelling-induced taurine release from NIH3T3 fibroblasts [61, 62]. Down-regulation of protein tyrosine phosphatase 1B in the cardiomyocyte renders the cell resistant to hypoxia/reoxygenation induced apoptosis, an effect attributed to either activation of Akt, reduction in caspase activity or diminished recruitment of tyrosine phosphatase 1B to FasR [63]. In this regard, it is significant that drug-induced taurine depletion also inhibits hypoxia-mediated apoptosis, an effect associated with the activation of Akt and the translocation of PKCε [57, 64]. However, it remains to be determined if the initiator of Akt activation is tyrosine kinase-mediated activation of PI 3-kinase or inhibition of protein tyrosine phosphatase 1B.

Besides playing a major role in cell survival, protein kinases also modulate myocardial contraction. Steele et al. [22] and Galler et al. [65] found that physiological concentrations of taurine modulate the calcium sensitivity of contractile proteins, an effect also seen in the drug-induced taurine deficient hearts [24]. The mechanism underlying this effect has not been directly examined, however, it is noteworthy that taurine depletion results in enhanced phosphorylation of troponin I [18]. Because troponin I phosphorylation interferes with the binding of calcium to troponin C, the elevation of troponin I phosphorylation would represent the mechanism by which taurine deficiency decreases calcium sensitivity of the muscle proteins.

Protein phosphorylation also modulates high energy phosphate metabolism. One of the metabolic enzymes phosphorylated in response to drug-induced taurine depletion is pyruvate dehydrogenase [65]. In the heart pyruvate dehydrogenase exists as a large multisubunit complex containing 3 enzyme activities, pyruvate dehydrogenase, dihydrolipoyl transacetylase and dihydrolipoyl dehydrogenase. Three serine residues of the pyruvate dehydrogenase enzyme are phosphorylated by pyruvate dehydrogenase kinase; pyruvate dehydrogenase phosphatase dephosphorylates the same 3 serine residues [67]. Pyruvate dehydrogenase kinase-mediated phosphorylation diminishes pyruvate dehydrogenase activity. The conclusion that pyruvate dehydrogenase exists in an inactive, presumably more phosphorylated state in the intact taurine depleted heart is borne out by studies showing that the
utilization of pyruvate/acetylCoA by the citric acid cycle is severely retarded, resulting in the accumulation of pyruvate and lactate by the taurine deficient heart [25].

Phosphorylation also plays a major role in cell signaling. The effects of taurine on cell signaling have been examined in the brain but not in the heart. Clearly additional experiments examining the effects of taurine depletion on cell signaling are warranted.

1.3. Therapeutic uses of taurine

Although taurine mediates several actions that could potentially benefit the diseased heart, taurine has been most widely used as a nutritional supplement. Nonetheless, wider acceptance of taurine as a therapeutic agent seems warranted. The present section discusses the potential use of taurine in the treatment of congestive heart failure, ischemic heart disease, Adriamycin toxicity and diabetic cardiomyopathy.

1.3.1. Therapeutic role for taurine in congestive heart failure

Taurine is presently approved in Japan for the treatment of congestive heart failure. The adoption of taurine as a therapeutic agent was largely based on convincing animal and human studies. In one of the important clinical studies, Azuma et al. [68] used a double-blind randomized crossover placebo controlled format to test the effect of taurine (6 g/day) on congestive heart failure patients (3 patients with class IV, 30 patients with class II and 29 patients with class II New York Heart Association classification). After 4 weeks of taurine treatment without any apparent adverse effects, significant improvements were noted in dyspnea, crackle, edema and New York Heart Association functional class. Taurine-mediated improvements were also achieved in patients treated with either digitalis or diuretics.

Prior to obtaining approval for the use of taurine as therapy in congestive heart failure, Azuma and coworkers [69-71] examined the effect of taurine on several animal models of heart failure. They found that oral administration of taurine (100 mg/kg/day) for a period of 8 weeks to rabbits with aortic regurgitation-mediated left ventricular failure significantly improved systolic function and reduced mortality [69]. Similarly, in an animal model of heart failure caused by administration of toxic doses of isoprenaline, administration of taurine (100 mg/day) decreased the degree of myolysis, interstitial edema, fibrosis, oxidative stress and calcium overload while improving the status of the adenine nucleotides and the phospholipids [70, 71]. Another heart failure model, the genetic BIO 14.6 strain of hamster, showed improvement
following taurine administration [72, 73]. Because a major lesion of the BIO 14.6 hamster heart is calcium overload, taurine’s action on cellular Ca^{2+} handling should prove beneficial in attenuating the severity of the calcium-linked lesions. Nonetheless, Keith et al. [74] reported that the heart of the cardiomyopathic hamster contains low levels of taurine, suggesting that the cardiomyopathic hamster may merely represent another taurine deficient heart failure model.

1.3.2. Treatment of ischemic heart disease with taurine

The worldwide WHO-CARDIAC study established a significant, inverse relationship between the population levels of taurine excretion and mortality from ischemic heart disease [75]. Although the relationship is largely related to the antihypercholesterolemia effect of taurine, a relationship remains despite normalization of the data relative to total serum cholesterol. Thus, taurine may benefit the ischemic heart through several mechanisms, including its antioxidant, calcium lowering and osmoregulatory actions.

Several models of ischemia-reperfusion have been developed. Because the outcome of an ischemia-reperfusion insult depends upon the model employed and the end point evaluated, careful attention must be paid to the details of each experiment. One of the well established and widely used methods of evaluating ischemia-reperfusion injury involves the measurement of infarct size/area at risk. In hearts subjected to regional ischemia, the area at risk represents the entire ischemic zone. Infarct size is determined from the volume of necrotic tissue, which is based on lack of triphenyltetrazolium chloride staining. Using this method, Allo et al. [76] found that drug-induced taurine depletion renders the heart resistant to ischemic injury. In apparent contrast to the Allo study, Ueno et al. [76] found that a short perfusion of hearts with buffer containing 10 mM taurine results in a significant decrease in infarct size, provided the short perfusion period is initiated either prior to the onset of global ischemia or immediately following the global ischemic insult. Despite the apparent contradictions between the two studies, it is noteworthy that the effects of taurine depletion and taurine treatment share certain properties, such as osmotic stress, which could explain the similarity in response. Pastukh et al. [57] described a phenomenon known as osmotic preconditioning, in which an osmotic insult initiated before the ischemia-reperfusion insult renders the heart resistant to the ischemia-reperfusion insult. Like other forms of preconditioning, osmotic preconditioning is initiated by the activation of the PI 3-kinase/Akt survival pathway and results in a significant reduction in infarct size. Both drug-induced taurine depletion
and taurine treatment activate the PI 3-kinase/Akt pathway by causing hyperosmotic stress [57, 78].

Other ischemia-reperfusion studies have utilized models and endpoints that focus on oxidative damage. Kramer et al. [79] developed a mild global ischemia model that results in myocardial stunning, a condition in which contractile function is depressed as a result of oxidative stress rather than cell death. We now know that taurine depletion exerts opposite actions on the more severe regional ischemia model and the mild myocardial stunning model, largely because of the relative importance of the PI 3-kinase/Akt pathway vs oxidative stress/energy metabolism in the two models [76, 80]. Taurine depletion activates the PI 3-kinase/Akt pathway and diminishes calcium overload, effects that contribute to the cardioprotection noted in the regional ischemic model [51, 79, 81]. However, taurine depletion also decreases the antioxidant and mitochondrial stabilizing actions of taurine, effects that dominate in the stunning model. By contrast, taurine treatment protects hearts subjected to the regional ischemia model and ischemic cells by activating the PI 3-kinase/Akt pathway [77, 82]. Recovery of mechanical function in the global ischemia model is minimally affected by taurine treatment [79, 83].

Ischemia-reperfusion leads to an inflammatory response involving the recruitment, adhesion and activation of neutrophils [84]. Because taurine scavenges HOCl, an oxidant produced by the neutrophil, it partially neutralizes the effects of neutrophils [38]. Therefore, it is not surprising that taurine treatment diminishes reperfusion injury and oxidative stress in hearts subjected to 15 min of global ischemia followed by reperfusion with medium containing neutrophils [85]. The possibility that taurine might diminish arrhythmias secondary to its antioxidant activity has also received some attention [86, 87].

The animal studies have prompted experiments evaluating the effect of supplementing St Thomas’ cardioplegic solution with taurine. Oriyanhan et al. [88] found that ischemic hearts treated with taurine supplemented cardioplegic solution exhibit less DNA oxidative stress and cell apoptosis. The beneficial effect of taurine has also been noted in patients undergoing bypass surgery, who exhibit less ultrastructural damage after receiving a rapid infusion of taurine (5 g) 1-3 hours prior to surgery followed by cold St. Thomas cardioplegic solution during surgery [89]. Because taurine is lost from ventricles of patients undergoing bypass surgery, it is logical to suggest that taurine treatment not only precondition the patient (by activating the PI 3-kinase/Akt pathway) but also minimizes damage caused by the loss of taurine from the heart [90].
1.3.3. Reduction in adriamycin-induced cardiotoxicity by taurine

Adriamycin is a potent antineoplastic agent, whose clinical use is limited by cardiac toxicity. The search for an effective antidote against the cardiotoxicity has been challenging, as Adriamycin’s toxic actions involve multiple mechanisms. One of those mechanisms results in the modification of DNA either through the formation of an Adriamycin-DNA adduct, elevation in DNA strand breaks or intercalation of DNA. Although these effects alter protein synthesis, it is difficult to imagine disruption of this mechanism by taurine, which exerts no known actions on DNA. Second, Adriamycin dramatically affects calcium handling by the cardiomyocyte. Among its effects are the enhancement of calcium current via the L-type Ca\(^{2+}\) channel, inhibition of the Na\(^+\)/Ca\(^{2+}\) exchanger, reduction in SR Ca\(^{2+}\) pump activity and promotion of calcium release from the sarcoplasmic reticulum. According to Hamaguchi et al. [91] and Harada et al. [92] Adriamycin-mediated elevations in myocardial calcium content are largely prevented by treatment with taurine while drug-induced taurine deficiency potentiates Adriamycin-mediated suppression of SR Ca\(^{2+}\) pump activity. These beneficial effects of taurine appear to be related in part to the improvement in SR Ca\(^{2+}\) pump expression, which is depressed by Adriamycin [93]. Third, Adriamycin promotes the generation of oxidants by microsomes and mitochondria. While taurine deficiency potentiates Adriamycin-mediated oxidative stress, taurine treatment diminishes it [92, 93]. Because calcium overload and oxidative stress are initiators of the mitochondrial permeability transition and apoptosis, one would predict that taurine might also attenuate Adriamycin-mediated apoptosis, an effect that could account for taurine-mediated reductions in the mortality of Adriamycin treated mice [91]. Despite these promising findings, further studies are warranted to evaluate the potential of taurine/Adriamycin co-therapy. In order for taurine to be considered for use as a co-therapy with Adriamycin, human studies must demonstrate that taurine significantly reduces Adriamycin cardiotoxicity without decreasing the anti-neoplastic activity of Adriamycin.

1.3.4. Potential therapeutic role for taurine in diabetes

Diabetes is a multifactorial disease associated with major complications, one being the development of a cardiomyopathy. Although the complications of diabetes are caused by either insulin deficiency (type 1 diabetes) or insulin insensitivity (type 2 diabetes), the pathology of the complications is similar in the two types of diabetes. The development of the diabetic cardiomyopathy
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has been largely attributed to events that are modulated by taurine, such as oxidative stress, impaired calcium movement and altered energy metabolism.

There is mounting evidence that diabetes is a disease of oxidative stress. In 2001, Brownlee [94] introduced the novel concept that glucose-mediated oxidative stress contributes to the development of diabetic complications. According to the Brownlee hypothesis, oxidants generated through glucose metabolism inactivate glyceraldehyde 3-phosphate dehydrogenase, resulting in the diversion of substrate away from the glycolytic pathway and increasing flux through pathological pathways, such as the polyol pathway, the hexosamine pathway, protein kinase C activation pathway and glycation end-product (AGE) formation (Figure 6). However, oxidants can cause direct damage to the heart without the involvement of the pathological pathways implicated in the Brownlee hypothesis [95, 96]. Because of the involvement of both the pathological pathway and direct oxidative damage to the diabetic heart, glucose-mediated oxidative stress is very damaging to the heart. Two taurine-containing conjugation reactions dramatically alter the effect of glucose-mediated oxidative stress. First, the amino group of taurine can form an amide bond with the carbonyl of reducing sugars. In the absence of taurine, these carbonyls accumulate in the diabetic heart, initiating a pathway leading to the formation of extensively cross-linked glycation end products. Not only are key proteins inactivated during the formation of the glycation end products, but the glycation end products also activate RAGE receptors [97] and stimulate the formation of ROS [98, 99]. Second, according to the Schaffer et al. [41], a large mitochondrial pool of taurine and of 5-taurinomethyluridine improves coupling between electron transport and ATP synthesis while lessening mitochondrial superoxide generation. Because the major source of glucose-mediated oxidative stress is the mitochondria and taurine improves mitochondrial coupling, a high taurine load would be expected to improve mitochondrial function, minimizing glucose-mediated oxidant formation by the mitochondria (Figure 6).

Impaired Ca\(^{2+}\) handling also contributes to the development of the diabetic cardiomyopathy. Based on calcium transients of nondiabetic and diabetic hearts, both peak [Ca\(^{2+}\)]\(_i\) and the rate of [Ca\(^{2+}\)]\(_i\) decline are significantly depressed in diabetic cardiomyocytes, with defects in SR Ca\(^{2+}\) handling central to both abnormalities [100-104]. A major anomaly in the diabetic heart is impaired expression of SR Ca\(^{2+}\) cycling proteins, such as the ryanodine receptor, Ca\(^{2+}\) pump ATPase and phospholamban [105-107]. Therefore, it is not surprising that overexpression of the SR Ca\(^{2+}\) ATPase improves contractile function and SR Ca\(^{2+}\) handling by the diabetic heart [108, 109]. Although taurine is unlikely to exert an effect on the expression of the SR linked proteins, taurine treatment should improve Ca\(^{2+}\) handling by
Figure 6. Attenuation by taurine of hyperglycemia-mediated activation of pathological pathways. In the Brownlee hypothesis, the metabolism of glucose by the mitochondria leads to the generation of superoxide, which feedback inhibits glyceraldehyde-3-phosphate dehydrogenase. This leads to the accumulation of glycolytic intermediates that precede the glyceraldehyde-3-phosphate dehydrogenase step. The accumulative effect of glucose, fructose-6-phosphate and glyceraldehyde-3-phosphate is a stimulation of pathological pathways, such as the polyol pathway, the hexosamine pathway and the glycation end product (AGE) pathway, that contribute to the development of diabetic complications. The elevation of glyceraldehyde-3-phosphate also promotes the synthesis of diacylglycerol, an activator of protein kinase C. Taurine reacts with the aldehydes of reducing sugars, preventing the accumulation of the AGE pathway. It also improves mitochondrial function, limiting the production of superoxide by cells exposed to high glucose.

by the diabetic cell. Holloway et al. [110] showed that addition of 20 mM taurine to the superfusate feeding diabetic cardiomyocytes significantly increases the amplitude and the rate of decay of calcium transients, actions expected for taurine treated hearts showing improved SR Ca^{2+} ATPase and the Na^{+}/Ca^{2+} exchanger activities. However, the possibility that taurine might benefit the diabetic heart by partially normalizing the activity of the SR Ca^{2+} release channels deserves further consideration [111, 112].

Diabetes is a metabolic disease characterized by impaired glucose metabolism and enhanced fatty acid metabolism. There is some evidence that taurine potentiates the actions of insulin [113]. However, the major effect of
taurine is to promote the synthesis of mitochondrial encoded proteins, thereby improving the function and coupling of the electron transport chain. Drug-induced taurine depletion diminishes normal mitochondrial function by interfering with the expression of mitochondria encoded proteins [41]. Because this slows respiratory chain activity, the taurine depleted heart also exhibits a reduction in pyruvate utilization by the citric acid cycle. Thus, superimposing the diabetic and taurine deficient phenotypes should lead to significant reductions in both glucose and fatty acid metabolism. On the other hand, taurine therapy should facilitate insulin action while enhancing the generation of ATP by both glucose oxidation and fatty acid metabolism.

1.4. Conclusion

Nearly 25 years have passed since Quinton Rogers and coworkers identified a dilated cardiomyopathy in cats containing low plasma levels of taurine. In those 25 years, little progress has been made in identifying the causes of the taurine deficient cardiomyopathy. Advancements in the field have been largely hampered by the limitations of the taurine deficient animal models. However, with the development of the taurine transport knockout model, meaningful mechanistic information should be forthcoming.

Several physiological actions of taurine could contribute to the development of the cardiomyopathy, including modulation of ion transport, antiapoptotic activity, antioxidant activity, osmoregulation and regulation of protein phosphorylation. The present review discusses the putative involvement of altered calcium movement, changes in protein phosphorylation and enhanced oxidative stress in the regulation of contractile function by taurine. However, the development of the cardiomyopathy is associated with ventricular remodeling, a process involving cell death and alterations in the size and shape of the cardiomyocytes. Based on its osmoregulatory activity, there is every reason to expect taurine to modulate the process of ventricular remodeling.

Only certain species develop the taurine deficient cardiomyopathy in response to nutritional taurine deficiency. These species contain ineffective biosynthetic pathways for taurine and lose large amounts of taurine as taurine conjugated bile acids. Humans do not readily synthesize taurine in the liver but form bile acid conjugates with both glycine and taurine, the former which protects humans against excessive loss of taurine. Nonetheless, the possibility that taurine deficiency might contribute to the development of cardiomyopathies in humans has not been adequately investigated. First, although the diet of most humans contains large amounts of taurine, it is
possible that polymorphisms might alter either the uptake or function of taurine. Second, there is evidence that certain drugs, such as Adriamycin, affect intracellular taurine levels. The present review discusses the possibility that Adriamycin cardiotoxicity might be related in part to taurine deficiency. Third, taurine is an accepted therapy for the treatment of congestive heart failure in Japan. A major advantage of taurine is that it mediates important actions without causing many side effects. Fourth, the present review suggests that taurine might be useful in the treatment of other disease conditions. Positive clinical outcomes have been reported for patients exposed to taurine containing cardioplegic solutions during coronary bypass surgery. There is also rationale for using taurine in the treatment of ischemic insults and diabetes. Fifth, taurine is a major component of energy drinks. Recent studies suggest that taurine might improve energy metabolism in heart and skeletal, providing an explanation for the improvement in endurance among the population that consume large quantities of energy drinks.

In the last 25 years, we have made major advances in understanding the actions of taurine. During the next 25 years we need to clarify the role of taurine in human physiology and pathology.

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1.6. References


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